



# Management of stocking density, pond size, starting time of aeration, and duration of cultivation for intensive commercial production of shrimp *Litopenaeus vannamei*

Javier M.J. Ruiz-Velazco<sup>a,b</sup>, Alfredo Hernández-Llamas<sup>c,\*</sup>, Víctor M. Gomez-Muñoz<sup>d</sup>

<sup>a</sup> Departamento de Desarrollo de Tecnologías, Centro Interdisciplinario de Ciencias Marinas del IPN, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23096, Mexico

<sup>b</sup> Dirección de Fortalecimiento de la Investigación, Universidad Autónoma de Nayarit, Cd. de La Cultura Amado Nervo s/n Tepic, Nayarit 63255, Mexico

<sup>c</sup> Programa de Acuicultura, Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23096, Mexico

<sup>d</sup> Departamento de Pesquerías y Biología Marina, Centro Interdisciplinario de Ciencias Marinas del IPN, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23096, Mexico

## ARTICLE INFO

### Article history:

Received 22 June 2010

Accepted 19 August 2010

### Keywords:

Farming management

Shrimp production

*Litopenaeus vannamei*

## ABSTRACT

A dynamic stock model was used for quantification of shrimp production and analysis of alternative management schemes of stocking density, pond size, starting time of aeration, and duration of cultivation for intensive commercial production of the shrimp *Litopenaeus vannamei*. Databases from Mexican farms were used to calibrate the model. Multiple linear regression models were employed to establish relationships between parameters of the stock model and the management variables. Water quality variables (dissolved oxygen, temperature, and salinity) were complementarily analyzed. The final weight of shrimp was directly related to duration of cultivation and dissolved oxygen, and inversely related to stocking density, pond size, and salinity. There were inverse relationships between the growth coefficient and temperature and dissolved oxygen and between mortality rate and temperature. Dissolved oxygen was significantly related to starting time of aeration. Simple linear regression and an equivalence test indicated that biomass at harvest (after 13 weeks in winter, and 20 weeks in summer) was adequately predicted by using the stock model and the multiple regression models. The highest production (winter, 6900 kg ha<sup>-1</sup>; summer, 12,600 kg ha<sup>-1</sup>) were predicted using 60 postlarvae m<sup>-2</sup>, small ponds (2 ha), and starting aeration at the first week of cultivation; while the lowest yields (winter, 2600 kg ha<sup>-1</sup>; summer, 6000 kg ha<sup>-1</sup>) were obtained using 40 postlarvae m<sup>-2</sup>, large ponds (8 ha), and delaying the start of aeration until the fifth week of cultivation. The lowest production was 38% (winter) and 48% (summer) of the highest yield. Using small ponds could be particularly important during winter cycles to increase production, while stocking density and starting time of aeration contributed less. In contrast, pond size played a minor role during summer cycles and stocking density was the most sensitive variable.

© 2010 Elsevier B.V. All rights reserved.

## 1. Introduction

In 2004, aquaculture activities were 43% of marine resource production worldwide. Shrimp was the most important product economically, being 17% of the global values of fishing products in international trade (FAO, 2007). According to CONAPESCA (2008), production of cultivated shrimp reached 130,200 tons in 2008, 66.33% of aquaculture production in Mexico.

There are many management factors that have been studied to increase production of cultivated shrimp. The literature on the

subject is very extensive and no attempt is made in this study to review this topic. Only a few investigations have focused on studying management variables acting simultaneously. Stocking density, duration of cultivation, and aeration are commonly investigated under controlled experimental conditions, rather than in commercial production units.

In a previous work (Ruiz-Velazco et al., 2010), using data from commercial farms, we studied the impact of the white spot disease on the dynamics of intensive production of *L. vannamei*, establishing relationships between parameters of a stock model and management and water quality variables. This demonstrated the important role that adequate management of stocking density, pond size and, particularly, starting time of aeration (i.e. the time when it is decided to turn on aerators for the first time, regardless the dissolved oxygen level) play in generally improving cultiva-

\* Corresponding author. Tel.: +52 612 123 8416; fax: +52 612 125 3625.

E-mail addresses: [marcialj@nayar.uan.mx](mailto:marcialj@nayar.uan.mx) (J.M.J. Ruiz-Velazco), [ahllamas04@cibnor.mx](mailto:ahllamas04@cibnor.mx) (A. Hernández-Llamas).

tion conditions and mitigating negative impacts of white spot disease.

From the literature review conducted for our present study, with the exception of our previous work, we did not find investigations dealing simultaneously with stocking density, pond size, and starting time of aeration for shrimp farming. The results obtained in that work led us to hypothesize that adequate simultaneous manipulation of those management variables, in addition to the duration of the cultivation period, should contribute to increased shrimp yields under normal operating conditions (that is, when not affected by white spot disease). Compared to other management variables, in this investigation, we were interested in determining the importance of starting time of aeration, a management variable that has not been previously analyzed for normal cultivation conditions of shrimp.

To fulfill our objective, we projected biomass yields of *L. vannamei* for alternative management schemes, using a stock model and functional relationships between its parameters and management and water quality variables. Databases from farms in the State of Nayarit were used for model calibration. Nayarit is the third largest producer in Mexico (CONAPESCA, 2008).

## 2. Materials and methods

### 2.1. Data survey

A database was prepared from data from two of the three intensive farms in Nayarit. The study units were 37 ponds (1.6–8 ha) used during winter (22), and summer (15) production cycles. The ponds were selected after confirming that shrimp was not affected by diseases, that there were not major operating problems and that biometrical, water quality and management variables were adequately monitored and registered.

For each pond, the following variables were analyzed: mean individual weight of shrimp (g), number of survivors, biomass yield (kg ha<sup>-1</sup>), initial stocking density (postlarvae m<sup>-2</sup>), pond size (ha), starting time of aeration (weeks), duration of cultivation (weeks), dissolved oxygen (mg l<sup>-1</sup>), water temperature (°C), and salinity (ppt).

According to farmers, weight of shrimp was measured weekly (0.01 g and 0.1 g precision balances, Ohaus, Pine Brook, NJ); cumulative survival of shrimp was weekly estimated by sampling with 1.5-m radius cast nets made of 3.2 or 25.4 mm square mesh, knotted monofilament line; DO and T were measured each day at 06:00 h and 18:00 h (oxymeter, Model 55, YSI, Yellow Springs, OH). S was measured weekly (refractometer, Aqua fauna Bio-Marine, Hawthorne, CA).

### 2.2. Stock model

Shrimp biomass ( $b_t$ ) at time  $t$  was calculated using a stock model:

$$b_t = w_t n_t \quad (1)$$

where  $w_t$  is mean individual weight of shrimp and  $n_t$  is the surviving shrimp population.

A modification of the Hernandez-Llamas and Ratkowsky (2004) model was used to describe growth of shrimp:

$$w_t = w_0 + (w_f - w_0) \left[ \frac{(1 - k^t)}{(1 - k^c)} \right]^3, \quad (2)$$

where  $w_0$  is the weight at the beginning of cultivation,  $w_f$  is the weight at the conclusion of the cultivation period,  $k$  is a parameter representing the rate at which  $w_t$  changes from  $w_0$  to  $w_f$ , and  $c$  is the duration or conclusion of the period of cultivation (weeks).

Survival was modeled using the exponential equation:

$$n_t = n_0 e^{-z \cdot t} \quad (3)$$

where  $n_0$  is the initial population, and  $z$  is the instantaneous mortality rate. Nonlinear regression estimation was used to fit the growth and survival curves to data corresponding to each pond.

### 2.3. Linear regression analyses

Relationships between parameters of the stock model and management and water quality variables were analyzed using multiple linear equations in the form:

$$Q = a_0 + a_1 D + a_2 PS + a_3 STA + a_4 DC + a_5 DO + a_6 T + a_7 S \quad (4)$$

where  $Q$  represents any of the stock model parameter:  $w_f$ ,  $k$ , or  $z$ , depending on initial stocking density ( $D$ ), pond size ( $PS$ ), starting time of aeration ( $STA$ ), duration of cultivation ( $DC$ ), dissolved oxygen ( $DO$ ), temperature ( $T$ ), salinity ( $S$ ), and  $a_0$ – $a_7$  are regression coefficients. The multiple regression models were fitted with datasets constituted by estimates of the stock model parameters and the corresponding values of management and water quality variables of all the ponds. The mean values of water quality variables measured during the cultivation period were used for analysis. The range of these mean values, together with the range of the management variables are presented in Table 1. Simple linear regression was used to determine relationships between dissolved oxygen and starting time of aeration.

Parsimony of the multiple regression models was achieved with two steps. Step 1 uses the backward stepwise procedure for selection of predictor variables. This procedure deals with colinearity with methods described by Rencher (2002). Step 2 follows Poole (1974), testing whether the functional relationships of the models were correct and that most of the significant factors affecting the parameters of the stock model were included.

For Step 2, the coefficients estimated from regression analyses were used to calculate the expected biomass at harvest for each pond and this was compared with the corresponding observed yield in the database. After this, a simple linear regression analysis between observed and expected shrimp biomass was performed, setting the intercept at zero. After determining that the regression slope did not differ significantly from 1 ( $t$ -test), an equivalence test (Chow and Liu, 2004) was conducted to protect against falsely not rejecting the corresponding null hypothesis (Type-II statistical error; Zar, 2010; Hauck and Anderson, 1986). The residuals resulting from this simple linear regression were tested for normality, using the Shapiro–Wilk  $W$ -test. For testing equivalence, tolerance error was set at 5% (Garret, 1997).

Software procedures available in Stata 10.0 (StataCorp, College Station, TX) and Statistica 6.0 (StatSoft, Tulsa, OK) were used to perform statistical analyses, setting significance at  $P < 0.05$ .

The stock model, together with the estimates of regression coefficients, was used to predict the dynamics of production for hypothesized alternative management strategies that considered the role of stocking density, pond size, and starting time of aeration. The extreme (minimum and maximum) values of the management variables and the mean values of water quality variables contained in the database were used for prediction.

The contribution of each management variable to the variation in biomass yields was calculated. The percentage of increase in biomass that could be achieved, when using the extreme values of the variable, was calculated for every combination of extreme values of the other management variables. The contribution of the variable was then calculated by determining the minimum, maximum, and mean percentages of increase. The higher the mean

**Table 1**  
Range of management and water quality variables used for multiple regression analysis. The maximum and minimum of the mean values of water quality variables during the cultivation periods are presented.

Variable	Winter		Summer	
	Maximum	Minimum	Maximum	Minimum
Stocking density (postlarvae m <sup>-2</sup> )	60	40	60	40
Pond size (ha)	8	1.6	8	1.6
Starting time of aeration (weeks)	5	1	5	1
Duration of cultivation (weeks)	13	10	15	20
Dissolved oxygen (mg l <sup>-1</sup> )	10.6	6.78	10.07	6.11
Temperature (°C)	29.8	26.37	32.12	31.13
Salinity (ppt)	35.34	31.05	27.38	17.25

value, the more important was the contribution of the variable for management.

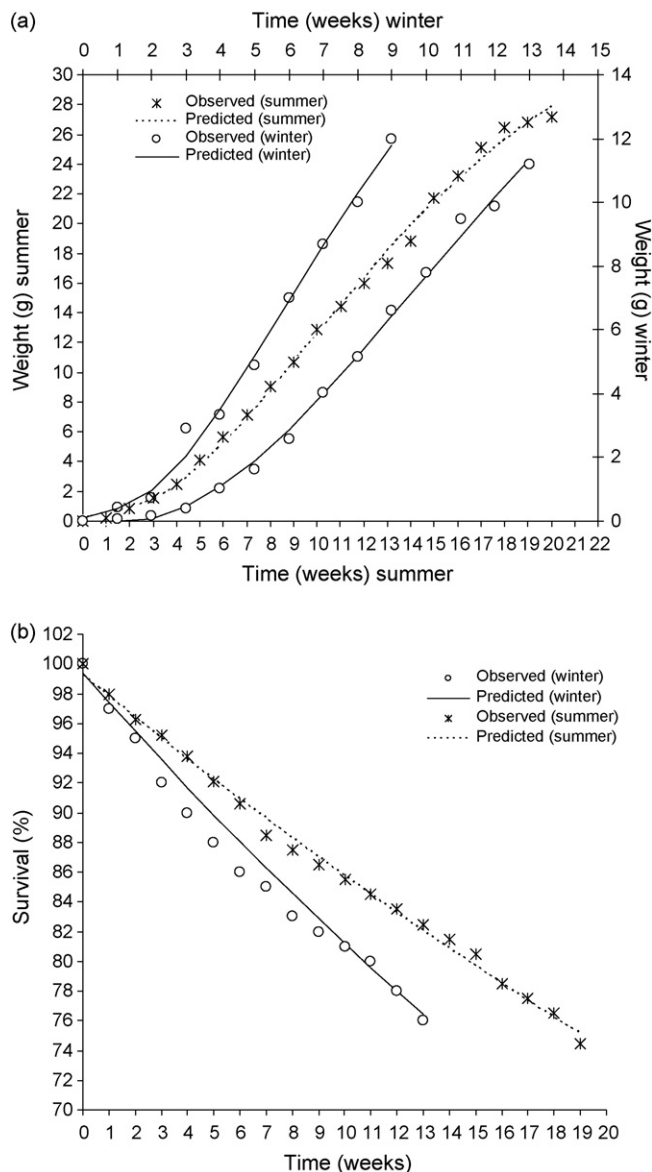
### 3. Results

By regression ANOVA, significant results were obtained when growth and survival curves were fitted to the datasets correspond-

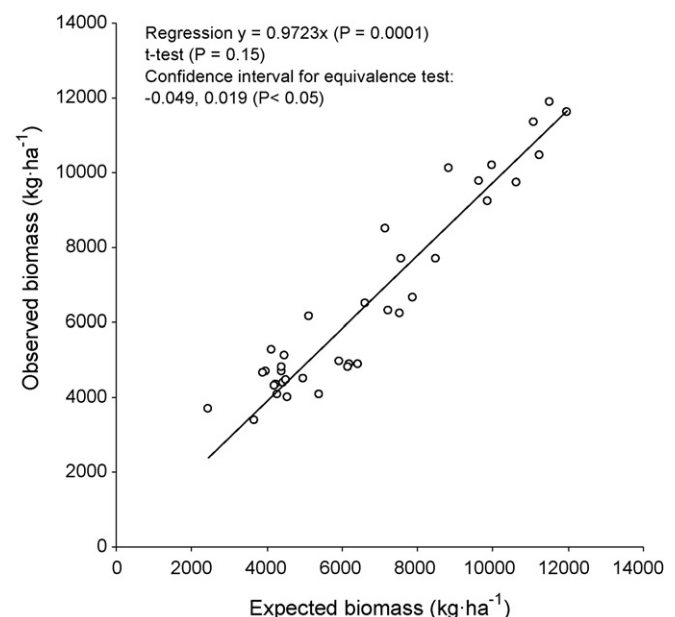
ing to all the ponds (Fig. 1). The growth and survival models showed flexibility to describe the different types of data trends.

Results from multiple regressions in Table 2 indicate that, with the exception of starting time of aeration, significant relationships existed between the parameters of the stock model and the management and water quality variables. The final weight of shrimp ( $w_f$ ) was directly related to duration of cultivation and dissolved oxygen, and inversely related to stocking density, pond size, and salinity. There were inverse relationships between the growth coefficient ( $k$ ) and temperature and dissolved oxygen and between the instantaneous mortality rate ( $z$ ) and temperature. Dissolved oxygen, in turn, was significantly related to starting time of aeration ( $DO = -0.068STA + 10.18$ ). Early start of aeration produced higher mean values of dissolved oxygen. This relationship was used to predict how  $w_f$  and  $k$  could be controlled by the starting time of aeration.

A significant relationship was obtained between biomass yields in the database and the yields calculated by the stock model, using the regression coefficients listed in Table 1 and the equations predicting dissolved oxygen as a function of starting time of aeration (Fig. 2). The regression coefficient did not differ significantly from 1 and equivalence between the coefficient and 1 was determined. Residual analysis did not indicate directional deviations from the fitted straight line and no evidence was found that the residual values were not normally distributed. We concluded that: (1) the functional relationships used were correct; (2) most of the sig-



**Fig. 1.** Example of fitted growth curves (a) two for winter and one for summer, and survival curves (b) one for each season, using the stock model.



**Fig. 2.** Relationship between observed and expected yields of biomass, using the stock model and the regression coefficients in Table 2.

**Table 2**

Regression coefficients used to predict parameter values of the stock model as a function of water quality and management variables.

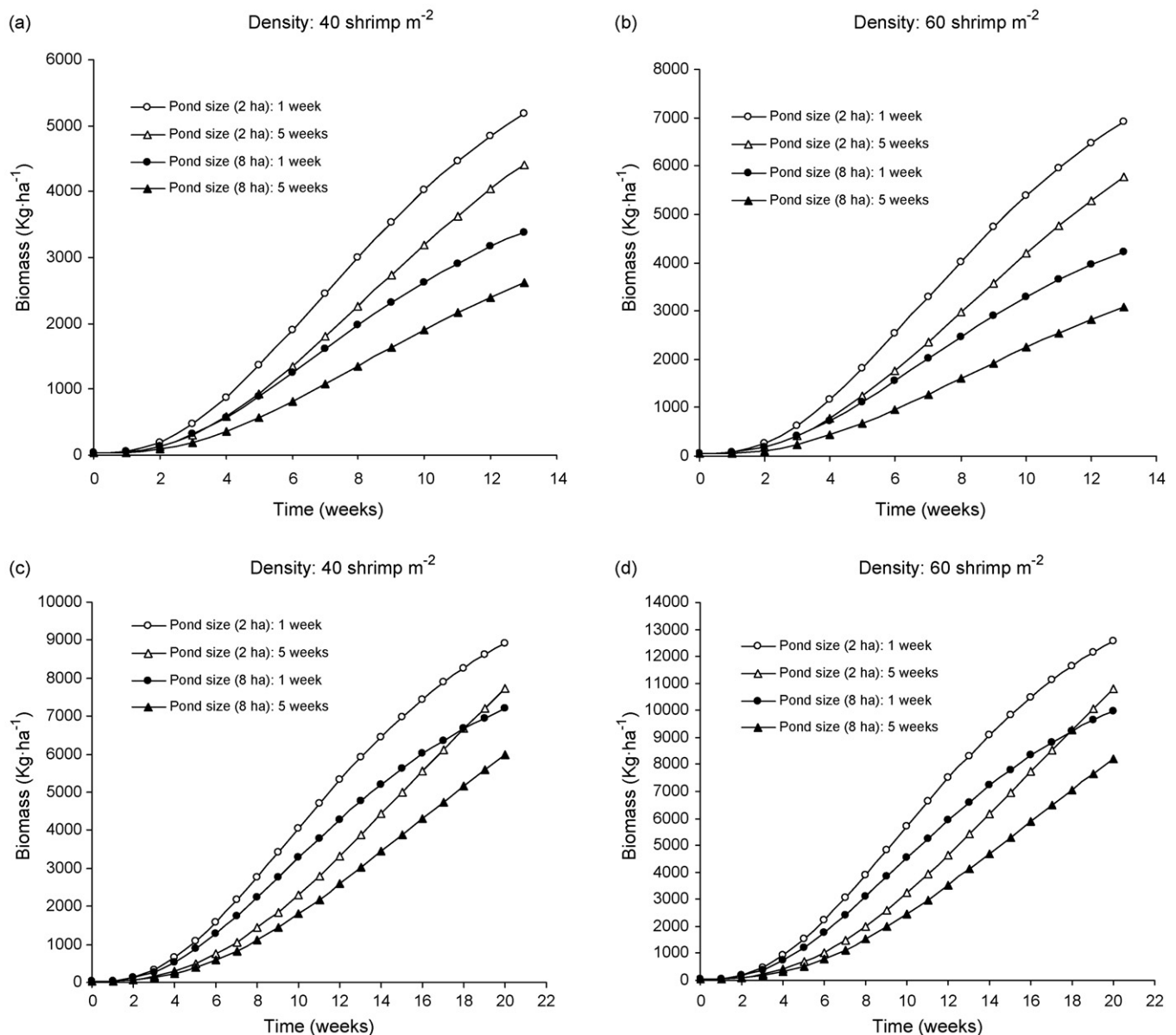
Parameter	Stocking density	Pond size	Duration of cultivation	Dissolved oxygen	Temperature	Salinity	Intercept
$w_f$	-0.091	-0.964	1.656	1.144		-0.235	-2.55
$k$			0.009	-0.012	-0.0063		1.03
$z$					-0.00096		0.04

nificant factors affecting shrimp biomass were included; and (3) there was no need for predictors other than those considered in Eq. (4).

Using the stock model to predict the dynamics of production showed that the highest yields would be obtained by employing the longest cultivation periods (13 weeks in winter and 20 weeks in summer). These cultivation periods were used to calculate yields. The highest productions (winter, 6900 kg ha<sup>-1</sup>; summer, 12,600 kg ha<sup>-1</sup>) were predicted using 60 postlarvae m<sup>-2</sup>, small ponds (2 ha), and starting aeration in the first week of cultivation (Fig. 3); while the lowest yields were predicted using

40 postlarvae m<sup>-2</sup>, large ponds (8 ha), and delaying the start of aeration until the fifth week of cultivation (winter, 2600 kg ha<sup>-1</sup>; summer, 6000 kg ha<sup>-1</sup>). Under these conditions, production is predicted to be 38% in winter and 48% in summer of the highest predicted yields.

The analysis of the contribution of each management variable to changes in biomass yields showed that pond size was particularly important during winter cycles (Table 3), while stocking density and starting time of aeration contributed less. In contrast, pond size played a minor role during summer production cycles, while stocking density was the most sensitive variable.



**Fig. 3.** Predicted dynamics of shrimp production as a function of stocking density, pond size, starting time of aeration, and duration of cultivation period during winter (a and b) and summer (c and d) production cycles.



**Table 3**

Contribution of the management variables to increases in shrimp biomass. Values for each variable are the minimum, maximum, and mean percentages of biomass increases obtained from the increases calculated for every combination of extreme values (within parentheses) of the remaining management variables. Mean values indicate the contribution of each variable.

	Minimum	Maximum	Mean
Winter			
Stocking density (40–60 postlarvae m <sup>-2</sup> )	17.8	33.7	26.87
Pond size (2–8 ha)	53.0	87.0	68.0
Starting time of aeration (1–5 weeks)	17.2	37.0	24.1
Summer			
Stocking density (40–60 postlarvae m <sup>-2</sup> )	36.4	40.8	38.9
Pond size (2–8 ha)	24.0	31.7	27.6
Starting time of aeration (1–5 weeks)	15.4	21.8	18.4

#### 4. Discussion

Our results show that there are important differences in biomass yields associated with management schemes for intensive production of *L. vannamei*. The use of small ponds during winter cycles is important because it allows farmers to obtain larger final weight of shrimp (Table 2). This helps compensate for the lower production that normally occurs during the winter compared with summer. High stocking density, on the other hand, appears to be the more convenient way to increase yields during the more favorable conditions of summer production cycles. Starting time of aeration played a minor role in determining shrimp production in both seasons.

The effect of stocking density on growth, survival, and yields of shrimp has been extensively studied. A negative effect of stocking density on performance of *L. vannamei* is usually reported (Wyban et al., 1987; Moss and Moss, 2004; Araneda et al., 2008). Our results also showed that growth was negatively affected by higher density, which resulted in lower shrimp production.

Kongkeo (1997), Milstein et al. (2005), and Islam et al. (2005) stress that small cultivation ponds allow better management and lead to increased growth, survival, yields, and economic returns of *Penaeus monodon*. Similar findings for *L. vannamei* are reported (Hernandez-Llamas and Villarreal-Colmenares, 1999; Magallon, 2006) and our study also indicates that, in general, greater final weight and biomass production of shrimp are obtained in smaller ponds. High sensitivity of production to final weight explains the importance of obtaining larger shrimp by using small ponds for winter cycles.

Aeration is commonly used to improve water quality in ponds and enhance production of farmed aquatic animals. Dissolved oxygen is a major factor in intensive production and it is directly related to aeration rate (Hopkins et al., 1991). McGraw et al. (2001), working with *L. vannamei* and *Litopenaeus stylirostris*, showed that increasing minimum dissolved oxygen by aeration resulted in higher survival, yield, and net income, although no significant differences occurred in final weight. We found a positive effect of higher dissolved oxygen on final weight and that dissolved oxygen, in turn, was controlled by the starting time of aeration.

In our previous investigation, we showed that early start of aeration delays and mitigates negative effects of white spot disease on cultivated *L. vannamei* (Ruiz-Velazco et al., 2010). Deposition of bottom sediment occurs gradually before aeration is started and, compared with an early start of aeration, a late start provokes more suspension of sediments and lowers mean dissolved oxygen. This leads to depressed respiratory activity and greater vulnerability in infected shrimp when dissolved oxygen is low. We found that, under normal operating conditions and contrary to what was initially hypothesized, starting time of aeration was of minor importance in controlling shrimp production, compared with other management factors. This reversal of expectations is most likely related to the reduced sensitivity of healthy shrimp to

low dissolved oxygen and poor water quality following removal of pond bottom sediments.

It is normally expected that biomass yield should consistently rise as the duration of cultivation increases. We calculated that the maximum biomass occurred when using the longest period of cultivation. Larger shrimp were obtained with longer cultivation, although no evidence was obtained on mortality (estimated as an instantaneous rate) being affected by this management factor.

It is generally recognized that shrimp growth is favored by higher temperature and lower salinity during summer cycles, compared with winter cycles. Our results indicate that higher final weight occurred when salinity declined. However, we did not find final weight of shrimp related to temperature. This is most likely a consequence of variance of final weight being adequately explained by the other water quality and management variables, thus making temperature a redundant predictor. Yet, a positive effect of higher temperatures on survival was detected. This is consistent with the findings of Ponce-Palafox et al. (1997) and Hennig and Andreatta (1998) who, working respectively with *L. vannamei* and for *Penaeus paulensis* found a positive influence of temperature on survival from 20 °C to 30 °C.

The interpretation of relationships between the growth coefficient  $k$  and water quality and management variables is not straightforward. When  $k < 1$ , the sigmoid-shaped growth curve is better defined. When  $k > 1$ , shrimp are growing exponentially. Sigmoid and exponential curves could be related to different operating conditions and there is no a priori reason to suppose that better or worse cultivating conditions necessarily lead to either type of growth curve. In our study, coefficient  $k$  is used merely for descriptive purposes and no attempt was made to interpret its values as a consequence of specific cultivating conditions.

Our modeling approach can be adapted to analyze cultivation conditions in regions other than the one studied in this investigation. Depending on what is hypothesized and anticipated this may require, using multiple regression analysis, the analysis of categorical variables or interaction between predictors. However, this may result in identifying confounding factors (e.g. seasons may obscure the role of temperature), or in an unmanageable number of possible interactions. We were mainly focused on studying the relationships between parameters of the stock model and management and water quality variables, and building parsimonious models. It was not necessary to study predictors other than those stated in Eq. (4) after finding that the functional relationships we used were correct, and that there was no evidence of exclusion of significant factors affecting shrimp biomass.

We concluded that maximization of biomass of *L. vannamei* can be obtained using the highest stocking density, the smallest ponds, the earliest start of aeration, and the longest duration of cultivation. Small ponds and high stocking density are particularly recommended for winter and summer cycles, respectively. Future research should focus on validating the prediction of yields associated with these recommendations and on determining, from an economic view, the optimal values of the management variables.

#### Acknowledgements

The authors express their gratitude to shrimp farmers in Nayarit for their cooperation. Editorial suggestions were provided by Ira Fogel of CIBNOR. J.M.J.R.V. was a recipient of doctoral fellowships from CONACyT and PROMEP.

#### References

- Araneda, M., Pérez, E.P., Gasca-Leyva, E., 2008. White shrimp *Penaeus vannamei* culture in freshwater at three densities: condition state based on length and weight. *Aquaculture* 283, 13–18.

- Chow, S.C., Liu, J.P., 2004. Design and Analysis of Clinical Trials: Concepts and Methodologies, 2nd ed. John Wiley & Sons, Hoboken, NJ.
- CONAPESCA, 2008. Anuario estadístico 2008. Comisión Nacional de Pesca y Acuicultura, <http://www.conapescasagarpa.gob.mx>.
- FAO, 2007. El estado mundial de la pesca y la acuicultura 2006. Department of Fishery and Aquaculture, Food and Agriculture Organization, Rome.
- Garret, K.A., 1997. Use of statistical tests of equivalence (bioequivalence tests) in plant pathology. *Phytopathology* 87, 372–374.
- Hauck, W.W., Anderson, S., 1986. A proposal for interpreting and reporting negative studies. *Stat. Med.* 5, 203–209.
- Hennig, O.L., Andreatta, E.R., 1998. Effect of temperature in an intensive nursery system for *Penaeus paulensis* (Pérez-Farfante, 1967). *Aquaculture* 164, 167–172.
- Hernandez-Llamas, A., Ratkowsky, D.A., 2004. Growth of fishes, crustaceans and molluscs: estimation of the Von Bertalanffy, Logistic, Gompertz and Richards curves and a new growth model. *Mar. Ecol. Prog. Ser.* 282, 237–244.
- Hernandez-Llamas, A., Villarreal-Colmenares, H., 1999. A software reference to shrimp *Litopenaeus* farming practices. *Aquacult. Econ. Manage.* 3, 267–280.
- Hopkins, J.S., Stokes, A.D., Browdy, C.L., Sandifer, P.A., 1991. The relationship between feeding rate, paddlewheel aeration rate and expected dawn dissolved oxygen in intensive shrimp pond. *Aquacult. Eng.* 10, 281–290.
- Islam, M.S., Milstein, A., Wahab, M.A., Kamal, A.H.M., Dewan, S., 2005. Production and economic return of shrimp aquaculture in coastal ponds of different sizes and with different management regimes. *Aquacult. Int.* 13, 489–500.
- Kongkeo, H., 1997. Comparison of intensive shrimp farming systems in Indonesia, Philippines, Taiwan and Thailand. *Aquacult. Res.* 28, 789–796.
- McGraw, W., Teichert-Coddington, D.R., Rouse, D.B., Boyd, C.E., 2001. Higher minimum dissolved oxygen concentrations increase penaeid shrimp yields in earthen ponds. *Aquaculture* 199, 311–321.
- Magallon, F.J., 2006. Desarrollo y aplicación de una metodología, para evaluar la variabilidad de la capacidad de carga de la acuicultura de camarón, en la región del Golfo de California. Doctoral Thesis. Universidad de La Habana, Havana, Cuba.
- Milstein, A., Islam, M.S., Wahab, M.A., Kamal, A.H.M., Dewan, S., 2005. Characterization of water quality in shrimp ponds of different sizes and with different management regimes using multivariate statistical analysis. *Aquacult. Int.* 13, 501–518.
- Moss, K.R.K., Moss, S., 2004. Effects of artificial substrate and stocking density on the nursery production of Pacific white shrimp *Litopenaeus vannamei*. *J. World Aquacult. Soc.* 35, 536–542.
- Ponce-Palafox, J., Martínez-Palacios, C.A., Ross, L.G., 1997. The effect of salinity and temperature on the growth and survival rates of juvenile white shrimp *Penaeus vannamei*. *Boone* 1931. *Aquaculture* 157, 107–115.
- Poole, R.W., 1974. An Introduction to Quantitative Ecology. McGraw-Hill, New York.
- Rencher, A.A., 2002. Method of Multivariate Analysis, 2nd ed. John Wiley & Sons, New York.
- Ruiz-Velazco, J.M.J., Hernández-Llamas, A., Gomez-Muñoz, V.M., Magallon, F.J., 2010. Dynamics of intensive production of shrimp *Litopenaeus vannamei* affected by white spot disease. *Aquaculture* 300, 113–119.
- Wyban, J.A., Lee, C.S., Sato, V.T., Sweeney, J.N., Richards, W.K., 1987. Effect of stocking density on shrimp growth rates in manure-fertilized ponds. *Aquaculture* 61, 23–32.
- Zar, J.H., 2010. Biostatistical Analysis, 5th ed. Prentice Hall, Upper Saddle River, NJ.